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Telomere dynamics in the first year of life, but not later in life, predict lifespan in a wild bird

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Abstract

Telomeres are protective, nucleoprotein structures at the end of chromosomes that have been associated with lifespan across taxa. However, the extent to which these associations can be attributed to absolute length vs. the rate of telomere shortening prior to sampling remains unresolved. In a longitudinal study, we examined the relationship between lifespan, telomere length and the rate of telomere shortening in wild, purple-crowned fairy-wrens (*Malurus coronatus coronatus*). To this end, we measured telomere length using quantitative polymerase chain reaction in the blood of 59 individuals sampled as nestlings and 4–14 months thereafter, and in 141 known-age individuals sampled on average three times across adulthood. We applied within-subject centring analyses to simultaneously test for associations between lifespan and average telomere length and telomere shortening. We reveal that the rate of telomere shortening and to a lesser extent telomere length in the first year of life independently predicted lifespan, with individuals with faster shortening rates and/or shorter telomeres living less long. In contrast, in adulthood neither telomere shortening nor telomere length predicted lifespan, despite a considerably larger data set. Our results suggest that telomere length measured very early in life (during development) and longitudinal assessments of telomere shortening during the first year of life constitute more useful biomarkers of total life expectancy than either telomere length measured after development, or telomere shortening later in adulthood.

KEYWORDS
Aves, biomarker, development, life expectancy, life history, longevity, pace-of-life, purple-crowned fairy-wren, qPCR

1 | INTRODUCTION

A principal aim shared by evolutionary ecology, conservation biology and biomedical research is to understand the physiological mechanisms underlying individual variation in lifespan. Telomeres are thought to be particularly important in this regard, and diverse lines of evidence have highlighted the potential for telomere dynamics to predict and link individual experiences with age, ageing

and lifespan (Monaghan & Haussmann, 2006; Tricola et al., 2018). Telomeres comprise highly conserved, noncoding DNA sequences and protein complexes that form protective caps at the ends of eukaryotic chromosomes (Blackburn, 2005). In the absence of high telomerase activity (Harley et al., 1990) (and alternative lengthening pathways; Lawlor et al., 2019) telomeres shorten with each round of somatic cell division and in response to diverse challenges (Watson et al., 2015). Once telomeres reach a critically shortened length,

cells enter a state of replicative senescence that is associated with age-related declines in organ function and death (Hornsby, 2003; Wong et al., 2003). A wide range of environmental stressors have been associated with interindividual variation in the rate of telomere shortening, presumably via oxidative stress pathways (Reichert & Stier, 2017, but see Boonekamp et al., 2017). While variation in telomere length prevents it from being an accurate marker of chronological age, telomere shortening may be a useful indicator of the life stress experienced by individuals (Epel et al., 2004; Kotrschal et al., 2007), and provide a biomarker of age-specific variation in biological age (i.e., individual state) and life expectancy (Houben et al., 2008; Monaghan, 2010).

Telomere length variation at any point after conception is due to a combination of initial telomere length in the zygote and changes thereafter. Hence, it is a challenge to verify to what extent associations with telomere length can be attributed to telomere length at sampling vs. telomere dynamics prior to sampling. Longitudinal studies (i.e., collecting repeat tissue samples and lifespan data from individuals of known age across their life course) are required to address this problem but these studies are difficult to conduct. Such difficulties are exacerbated in natural conditions because dispersal from study populations can confound measures of lifespan. Yet, the study of natural populations is important because the fitness consequences (lifespan) of a trait (telomere shortening) are best estimated under the circumstances where animals face the adversities that have shaped their evolution. Most investigations into the relationship between lifespan and telomere dynamics in the wild have been based on cross-sectional analyses (Eastwood et al., 2019; Haussmann et al., 2005; Tricola et al.,2018; Wilbourn et al., 2018) which do not separate within-individual effects (i.e., the rate of telomere shortening) from population-level processes or prior selection events (such as the selective disappearance of poor-quality individuals with age), which may produce sample biases (Salomons et al., 2009). Several "telomere–lifespan" studies have collected longitudinal telomere length data but have used cross-sectional approaches to investigate links between telomere length and lifespan (Bichet et al., 2020; Pauliny et al., 2006; van Lieshout et al., 2019; Vedder et al., 2021; Watson et al., 2015). Consequently, the question of the relative importance of absolute telomere length and telomere shortening in causing associations between telomere length and lifespan is largely unresolved.

A limited number of studies have used longitudinal telomere data to assess "telomere shortening – lifespan" associations, but only track a binary measure of survival (survived or not) for a relatively short period after sampling, and thus do not assess lifespan per se (Boonekamp et al., 2014; Froy et al., 2021; Salomons et al., 2009; Wood & Young, 2019). For example, it has been shown that the rate of telomere shortening during the nestling phase (Boonekamp et al., 2014; Wood & Young, 2019) and during later life stages (Salomons et al., 2009) is accelerated in birds that do not survive to the following year compared to those that do. Thus, while these studies indicate that individuals facing relatively imminent mortality have higher rates of telomere shortening compared to the remaining surviving

population, they do not clarify whether the rate of telomere shortening predicts future life expectancy consistently throughout life. To our knowledge, only two longitudinal investigations address this question (Barrett et al., 2013 and Heidinger et al., 2012), and their results are mixed. One of the studies, on wild Seychelles warblers (*Acrocephalus sechellensis*) at the adult life stage (>1 year) (Barrett et al., 2013), used within-subject centring to assess longitudinal and cross-sectional telomere data and showed that the rate of telomere shortening during adulthood was negatively related to lifespan, but absolute telomere length was not. The other study, on captive zebra finches (*Taeniopygia guttata*) at the early life stage (25 days to 1 year) (Heidinger et al., 2012), assessed whether lifespan was influenced by the fixed effects "telomere length change between 25 days and 1 year" and "telomere length (at 25 days and 1, 3, 4, 6 and 7 years)" and found no association between telomere shortening and lifespan, but found a strong, positive association between telomere length at 25 days and lifespan. Differences in the results of these studies could reflect environmental effects on telomere shortening rates, which are presumably diminished in captive compared to wild populations. Or, because telomere shortening rates are generally higher in early life compared to adulthood (Benetos et al., 2009; Salomons et al., 2009), the different life stages over which shortening has been assessed in these studies may have obscured patterns relating to lifespan. Further work, in natural populations, is required to assess whether individuals with accelerated rates of telomere shortening are associated with a reduced life expectancy, and whether this potential relationship is dependent on the life stage at which the rate of telomere shortening is assessed.

In our longitudinal study, we examined life-long telomere dynamics in wild purple-crowned fairy-wrens (*Malurus coronatus coronatus*), a small long-lived songbird, from early life (the time of near complete skeletal growth [age 7 days]) and at various points thereafter (~1–10 years of age). We used a high-quality, long-term data set (>15 years) including lifespan and complete life-history information for several cohorts. These data enabled us to examine the relationship between telomere length, rates of telomere shortening at different life stages and individual lifespan. Our approach involved a statistical model that allowed us to simultaneously test the association of lifespan with telomere length and telomere dynamics. Previous research in this population showed that nestling telomere length predicts lifespan in birds that survived their first year (Eastwood et al., 2019). Here we investigate whether the rate of telomere shortening across early and later life may also explain variation in age of death in this wild bird population.

2 | MATERIALS AND METHODS

2.1 | Study species and population

Purple-crowned fairy-wrens are small (~12 g) riparian habitat specialists endemic to the monsoonal savannahs of tropical far-north Western Australia. At our site, they are restricted to riparian

vegetation dominated by *Pandanus aquaticus*, a palm-like monocot tree, which is used for nesting, foraging and roosting. We studied a population of purple-crowned fairy-wrens at the Australian Wildlife Conservancy's (AWC) Mornington Wildlife Sanctuary (126.1°E, −17.5°N), along 15 km of Annie Creek and Adcock River (core study area). All individuals have been uniquely colour-banded and routinely monitored since 2005 as part of a long-term research project, providing accurate information on survival and lifespan of each individual from hatching or immigration into the population (Roast et al., 2020). Purple-crowned fairy-wrens are cooperatively breeding birds that live in social groups, consisting of a dominant breeding pair and zero to eight subordinates. The groups defend year-round stable territories which are distributed linearly along water courses (Kingma et al., 2011). Breeding pairs, identified by duet song (Hall & Peters, 2008, 2009), are monogamous (with <5% extra-pair paternity; Kingma et al., 2013) and can breed all year round with a peak in breeding activity during the wet season (December–March) (Hidalgo Aranzamendi et al., 2019). Subordinate males—often retained previous offspring—usually acquire a breeding position on their natal territory or by taking up a vacancy nearby on average around 1.5 years of age (Hidalgo Aranzamendi et al., 2016). In our population, maximum recorded lifespan is 14.5 years (our unpublished data).

2.2 | Field sampling

From 2006 to 2010 we monitored territories year-round by weekly visits to record the presence and social status of all individuals, and document nesting activity throughout the year (whereby most nesting took place during the Wet season breeding season); in 2016–2018, we only monitored breeding during the Wet season (December–April). Nestlings were banded on day 7 from hatching (nestlings fledge at ~14 days) and a blood sample from the brachial vein was collected in a heparinized capillary tube which was then centrifuged. The plasma was removed and red blood cells were stored in Longmire's lysis buffer or ethanol. Blood samples were stored at 4°C for a period of 0.06–10.1 years prior to telomere quantification (see below). Sex was determined using an established PCR (polymerase chain reaction) protocol, validated in adults by sexually dichromatic plumage (see Fan et al., 2017). We sampled free-flying birds throughout the year from 2006 to 2010, and took blood samples as above. From 2011, complete censuses of the core study area were conducted before and after the Wet season to record the presence of all individuals. We also captured and blood sampled previously banded birds whilst new captures were banded and blood sampled (fledglings from unknown nests or dispersers) (Hidalgo Aranzamendi et al., 2016; Kingma et al., 2009, 2013); the parentage of newly captured birds was confirmed genetically (for details see Hidalgo Aranzamendi et al., 2016).

The estimated census detection rate of individuals per sampling season in the core population is 98%. Immigration into the study area is ~11% (78/691 birds >1 year are immigrants). To minimize errors in

lifespan estimates due to emigration, we conducted intensive yearly censuses from 2007 onwards to find birds that had dispersed outside the core area. This species is known to disperse primarily along waterways, and ~95 km of suitable habitat along ~200 km of waterways is surveyed annually, discovering emigrants that dispersed from the core banded population (Roast et al., 2020). The accuracy of our census technique is extremely high (>90% detection) due to the targeted, playback-based search technique and the species' habitat specificity (Hidalgo Aranzamendi et al., 2016). Fewer than 1% of birds assumed to have died were resighted inside or outside the core study area (Eastwood et al., 2019). No bird from our population has ever been resighted beyond the range of our census, and dispersal outside the river vegetation has never been recorded for this species (Skroblin et al., 2014; Skroblin & Legge, 2010). However, since landscape-level surveys were only conducted annually, estimates of lifespan are less accurate for birds that emigrated from the core study area, and these are not included in the study.

2.3 | Telomere analyses

We used quantitative PCR (qPCR) to analyse relative telomere length (rTL), with GAPDH as a reference, following protocols described in Eastwood et al. (2018), Eastwood et al. (2019). Briefly, DNA was extracted from red blood cells using a QIAamp DNA kit and QIAcube HT instrument (Qiagen) with protocol modifications as per Eastwood et al. (2018). All DNA samples (stored in AE buffer for up to 4 weeks at 4°C and at −20°C; defrosted at most once if required) were checked for purity and concentration (using a NanoDrop ND-1000), and for integrity on a 1% agarose gel prior to qPCR. qPCR mixes were automatically prepared using an EpMotion 5075 on 96-well plates. The qPCR method was originally based on Reichert et al. (2015) and validated for use in purple-crowned fairy-wrens (Eastwood et al., 2018). In each well, the reaction mix totalled 25 μl, including 12.5 μl of Lightcycler 480 SYBR Green I Master (Roche, product number 04887352001), 300 nM of both the normalizing control gene (glyceraldehyde-3-phoshate dehydrogenase; GAPDH) primers (Atema et al., 2013), 400 nM of both telomere primers (Integrated DNA Technologies) (Reichert et al., 2015) and 10 ng of sample DNA or 10 ng of an interplate control sample (a pool of purple-crowned fairy-wren DNA). All samples and controls were run in duplicate on each plate, for both telomere and GAPDH amplification using a LightCycler 480 (Roche) machine as follows: telomere (95°C for 15 min, followed by 35 cycles of 15 s at 95°C, 30 s at 56°C, 30 s at 72°C) and GAPDH (95°C for 15 min, followed by 40 cycles of 15 s at 95°C, 30 s at 60°C, 30 s at 72°C). rTL was calculated following Eastwood et al. (2019). Correct product amplification and qPCR quality was assessed by visualizing melt-curves and the no-template control (nuclease-free water). Average qPCR efficiencies were 91.64 (SD 5.9) for the telomere assay and 97.27 (SD 5.90) for GAPDH, and were calculated from a two-fold serial dilution starting at 40 ng of DNA from the control sample. A validation study previously

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confirmed high intraassay Cq repeatabilities (intraclass correlation coefficients were 0.91 [telomere] and 0.97 [GAPDH]) and high rTL repeatability (interassay = 0.85 and interextraction = 0.88 using this method in purple-crowned fairy-wrens; Eastwood et al., 2018).

The qPCR method does not distinguish between terminal telomeres and interstitial telomeric sequences (ITS), which vary strongly in number between species (Foote et al., 2013). rTL is an estimate of these different types of telomeres combined, which complicates interpretation of rTL data. We therefore investigated the frequency of ITS in 39 samples by comparing denatured and nondenatured TRF gels (Figure S1); details on samples, sampling protocol and TRF protocol are given in Eastwood et al. (2018), and gels were denatured following Foote et al. (2013), including a positive control on the gel (a sample of a species with many ITS). Because denaturing enables binding of the telomere probe to the ITS in addition to the terminal telomeric sequence, the appearance of new bands or sections of binding indicates the presence of ITS. Visual inspection of the difference between the nondenatured and denatured gels (Figure S1) showed there to be very few ITS in the purple-crowned fairy-wren samples, supporting our implicit assumption that rTL variation primarily reflects variation in the length of terminal telomeres. The high (0.74, Eastwood et al., 2018) correlation between qPCR estimates of telomere length and TRF also attests to the low influence of ITS on telomere estimation by qPCR.

2.4 | Statistical analysis

Statistical analyses were conducted in R 3.6.2 (R Core Team, 2021). rTL values were z-transformed (mean centred and scaled to one standard deviation [SD]) prior to analysis to improve the comparability of our results to other qPCR-based rTL studies (Verhulst, 2020). All age factors (lifespan and age [days]) were log transformed prior to analysis because this improved model fit considerably when assessed using Akaike's information criterion (AIC) (early life model with log-transformed age factors, AIC = 302.8, compared with untransformed age, AIC = 372.0, later life model with log-transformed age factors, AIC = 1156.4, compared with untransformed age, AIC = 1297.9). To investigate the relationship between lifespan and individual rTL changes during early and later life, two linear mixed models (LMMs) were run with rTL as the dependent variable. Sampling ages were chosen to encompass life-history and survival pattern differences between an early life stage with high mortality rates (mortality is highest within the first year at 70%), and later life adult stages with low mortality rates (mortality drops to 35% during the second year and 18% from the third year; our unpubl. data) and reproduction (individuals acquire a breeding position on average at 1.5 years; Eastwood et al., 2019). Furthermore, an earlier analysis showed that the association between lifespan and telomere length in nestlings changed with age, being strong for individuals that survived their first year, but much weaker when including birds that died in the first year of life. Sampling ages were restricted to

provide a sufficient interval to detect potential rTL changes (minimum 6-month interval). LMMs were run using the function lmer implemented in the package LME4 (Bates et al., 2015) using restricted maximum-likelihood estimates of the parameters.

In our first model we tested the cross-sectional and longitudinal association between lifespan and individual TL during the first year of life. Individuals (n = 118 rTL measurements from 59 individuals, from 38 unique parental pairs, i.e., two measurements per individual) included in this analysis were (i) sampled for rTL at the nestling stage (between 4 and 11 days, average = 6.0 days, SD = 1.1), (ii) sampled for rTL again, later in their first-year of life (between 4 and 14 months, average 244 days [SD = 68], range = 130–415 days) and (iii) were deceased at the time of analysis to provide an individual lifespan measure.

In our second model we tested the cross-sectional and longitudinal association between lifespan and rTL during later life, using a data set that was complementary to the data set used for the first model. Individuals (n = 425 rTL measurements from 141 individuals with 97 unique parental pairs) included in this second LMM were (i) sampled for rTL between 4 and 14 months (average age 284 days, SD = 91, range = 131–436 days), (ii) sampled for rTL again, at one or more other time points >6 months from the initial rTL measure between 4 and 14 months (age between 340 and 3917 days, average = 1213 [SD = 770]), and (iii) were deceased at the time of analysis to provide an individual lifespan (days) measure. In the data set for our second model, individuals were sampled between one and 10 times (average 2.5 later-life rTL measure repeats, SD = 1.8). Intervals between the age at which an individual was initially measured during the later stages of their first year and the later life measures was 895 days (SD = 762, range 171–3674 days).

In both models, rTL was the dependent variable, and we used within-subject centring, where an individual's age at sampling was partitioned into "average" and "delta age" which were both included as explanatory variables. An individual's average age was calculated as the average of all sampling ages at which an individual's rTL was measured, while delta age was calculated as the difference between an individual's age at measurement and its average age (i.e., delta age = age–average age) (van de Pol & Wright, 2009). Importantly, this analysis identifies significant differences in within-individual (delta) and between-individual (average) patterns, and hence whether data are confounded by population-level effects such as selective disappearance or cohort effects (van de Pol & Verhulst, 2006; van de Pol & Wright, 2009). To investigate the potential relationship between individual telomere rate of change (as opposed to absolute telomere length) and lifespan we included lifespan and its interaction with delta age (within-individual effect) as an explanatory variable in both models. In doing so, our models allow us to simultaneously assess the effect of absolute rTL and individual change in rTL over time on lifespan. "Sex" and "storage medium" (Longmire's buffer or ethanol, for rationale see Eastwood; Mulder; Verhulst, 2020) were included as fixed effects in both analyses. Since storage duration of blood may affect telomere measurements in birds (Reichert et al., 2017), we tested for effects of blood and DNA sample storage duration

by including "Days between sampling and DNA extraction (*z* transformed)" and "Days between DNA extraction and telomere quantification (*z* transformed)" in the models. We included. "*qPCR run ID*" as a random term to account for variation between qPCR runs. "*Individual ID*" was included as random term to reflect the repeated sampling, and "full sibling identification (*Full sibling ID*)" to account for the dependence of nestlings that shared the same genetic ancestry (the same mother and father) and the same early life environment. Since telomere dynamics can differ among cohorts within the same population (Spurgin et al., 2018), cohort ("*Hatch year*") was also included as a random effect.

3 | RESULTS

3.1 | First year of life

Across the first year of life, from the nestling period onwards, rTL shortened at an average rate of 1.115 rTL units per year (SD = 0.053). However, telomere shortening rates ranged widely, between −0.011 and 0.015 rTL units per day (Figure 1a). Over the early life period, 45 individuals showed a decrease in rTL and 14 individuals an increase in rTL, which we attribute to measurement error combined with the relatively short time interval between measurements (Steenstrup et al., 2013, but see Hoelzl, Cornils, et al., 2016; Hoelzl, Smith, et al., 2016 for evidence of telomere elongation in a hibernating rodent). As expected, on average individual rTL declined significantly with age, as evident from the significant effect of delta age (within-individual differences in sampling age) across early life (Table 1). Note that age was log-transformed, implying that this decline was decelerating. A significant, positive relationship between rTL and the interaction between lifespan and delta age was detected. This means that the negative relationship between rTL and within-individual (delta) age was significantly dependent on lifespan (i.e., the reduction in rTL across early life was reduced in individuals with long lives). This result indicates that the rate of rTL shortening across early life was accelerated in individuals with a shorter lifespan (Table 1, Figure 2).

A significant, positive relationship between rTL and lifespan was detected (Table 1). Average age (i.e., between individual differences in sampling age) did not impact rTL, suggesting no selective disappearance according to individual variation in rTL at this life stage. Sex, type of storage medium, and storage duration of both the blood and DNA samples were not significantly associated with rTL.

3.2 | Later life

From later in the first year to later adulthood, rTL shortened at an average rate of 0.043 rTL units per year (SD = 1.184), more than 25 times slower than in the first year of life (1.115 rTL units per year) (Figure 1b). In total, 82 individuals showed a decrease in rTL and 68 individuals an increase in rTL over the measurement period (Figure 1b). In this sample, individual TL was negatively, but not significantly, associated with delta age. We note that the slope of the delta age effect was very similar in the two analyses, but because age was log-transformed this still translates to a slower rate of telomere shortening in later life (i.e., after the first year). rTL was not associated with the interaction between lifespan and delta age; that is, the rate of rTL shortening across later life was not associated with lifespan (Table 2, Figure 2b). Additionally, rTL was not associated with lifespan, buffer, average age, or blood or DNA storage duration.

4 | DISCUSSION

Telomere length predicts survival in many species (Wilbourn et al., 2018). Here, we investigated to what extent this association can be attributed to telomere shortening and/or absolute telomere length in wild, purple-crowned fairy-wrens by examining the relationship between lifespan and longitudinal measures of telomere length. We show that a higher rate of telomere shortening in the first year of life, from the nestling stage to early adulthood, strongly predicted a reduced lifespan. However, among birds in adulthood we did not detect an effect of telomere shortening on lifespan. We did, however,
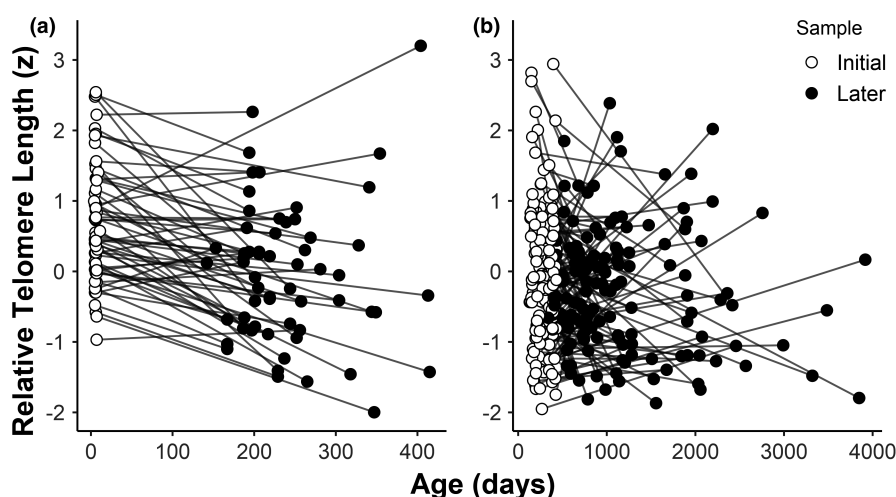

FIGURE 1 Individual relative telomere length (*z*-transformed) across (a) from the nestling stage to later in the first year and (b) later in life. Repeat samples from the same individual are connected by lines, with open symbols indicating the first sample and closed symbols indicating the last sample; for illustrative purposes in graph (b) only the first and last telomere measures are included even for individuals with more than two telomere measures

TABLE 1 Telomere length in the first year of life in relation to age and lifespan

Dependent variable: Relative telomere length (rTL(z))			
Fixed effects	**Estimate (SE)**	**t-value**	**p-value**
Intercept	2.020 (2.397)	0.843	.403
Lifespan	0.497 (0.245)	2.031	.049*
Lifespan × Delta age	0.585 (0.183)	3.190	.003*
Delta age	−2.031 (0. 529)	−3.837	<.001*
Average age	−2.017 (1.513)	−1.333	.188
Sex (male)	−0.090 (0.174)	−0.515	.609
Storage medium (Longmire)	0.630 (0.664)	0.950	.345
Days between sampling and DNA extraction (z)	−0.502 (0.316)	−1.588	.119
Days between DNA extraction and telomere quantification (z)	0.302 (0.948)	0.318	.751
Random effects	**Variance (SD)**		
Individual ID	0.085 (0.291)		
qPCR run ID	0.185 (0.430)		
Full sibling ID	0.261 (0.511)		
Hatch year	0.053 (0.231)		
Residual	0.261 (0.511)		
	Marginal R^2	**Conditional R^2**	
	0.199	0.753	

Note: *Significant effects.

All age parameters are expressed in log(days) and were log-transformed prior to the calculation of average and delta age. Note that because delta age was mean centred, the estimate of the lifespan effect remains informative despite being included in an interaction term.

Abbreviations: SE, standard error; SD, standard deviation.

find a trend for a positive effect of average telomere length in the first year of life on lifespan, independent of telomere shortening, whereas this effect was not detected among birds in adulthood. Below we discuss these results in turn.

4.1 | Telomere shortening and lifespan

Telomere length shortened significantly across the first year of life but did not change significantly across adulthood. In accordance with these results, several other studies on mammalian and avian species have also found that telomere loss is greatest during early life (Barrett et al., 2013; Foote et al., 2011; Pauliny et al., 2006; Salomons et al., 2009; Zeichner et al., 1999). What causes higher rates of telomere shortening during the first year remains to be established, but probably includes the cumulative impact of greater challenges earlier in life (Herborn et al., 2014), for example growth

and high metabolic rates, activated oxidative stress pathways in combination with immature antioxidant and DNA repair systems, as well as stress from skill learning during maturation and competition for independent breeding positions (Monaghan, 2014).

High rates of telomere shortening during the nestling stage have been shown to predict poor survival prospects (i.e., a reduction in the likelihood of surviving to the first year) in several avian species (Boonekamp et al., 2014; Wood & Young, 2019). However, this is the first demonstration that early life telomere shortening (from the nestling phase to 4–14 months) is predictive of total lifespan in a wild animal population, and it would be of interest to establish whether the same mechanisms cause survival effects on different timescales. Thus, in addition to predicting relatively "imminent" mortality, the rate of early life telomere shortening is also predictive of survival in the long term. This observation may be contingent on the study of wild vs. captive populations, as a similar longitudinal study on captive birds (Heidinger et al., 2012) did not detect a relationship between lifespan and early life telomere shortening (from 25 days to the first year of life). This contrast could reflect early life environmental effects on telomere shortening rates (Watson et al., 2015), which are presumably diminished in captive compared to wild populations. It is well established that early life experiences may impose long-term fitness consequences, and our results, from a wild population, support the view that the telomere shortening rate in the first year of life may act as a biomarker for differences in lifespan that can arise among individuals reared under different conditions.

The observed negative relationship between lifespan and the rate of telomere shortening during the first year of life may reflect a causal (i.e., animals die because their telomeres reach a critical length) and/or a noncausal effect (i.e., factors that influence telomere shortening also damage other processes related to lifespan). In our study, longevity was predicted by telomere shortening in the first year of life (before senescence is likely to occur) but not later in life (when senescence is more likely to occur). These results suggest that telomere shortening more probably reflects a noncausal biomarker of lifespan in our species—chronicling the stress of an individual's early life experiences, rather than being a direct cause of reduced lifespan. In other words, because the rate of telomere shortening during *early* life predicts longevity (probably before telomeres would have reached detrimentally short lengths), but not telomere shortening in *late* life (when telomeres might be reaching critically short lengths), the relationship between shortening rate and lifespan more probably reflects a mutual relationship with third-party mortality factors rather than shortening rate having an active role in determining an individual's age of death. In line with this, numerous factors experienced during early life, such as brood size/competition (Costanzo et al., 2016; Nettle et al., 2013; Reichert et al., 2014; Voillemot et al., 2012) and ambient temperature (Fitzpatrick et al., 2019; Stier et al., 2020) among others (Injaian et al., 2019; Kotrschal et al., 2007; Salmón et al., 2016), that affect telomere length dynamics are also known to affect fitness outcomes such as lifespan (Monaghan, 2008). Thus, although determining causality of associations between telomeres and age
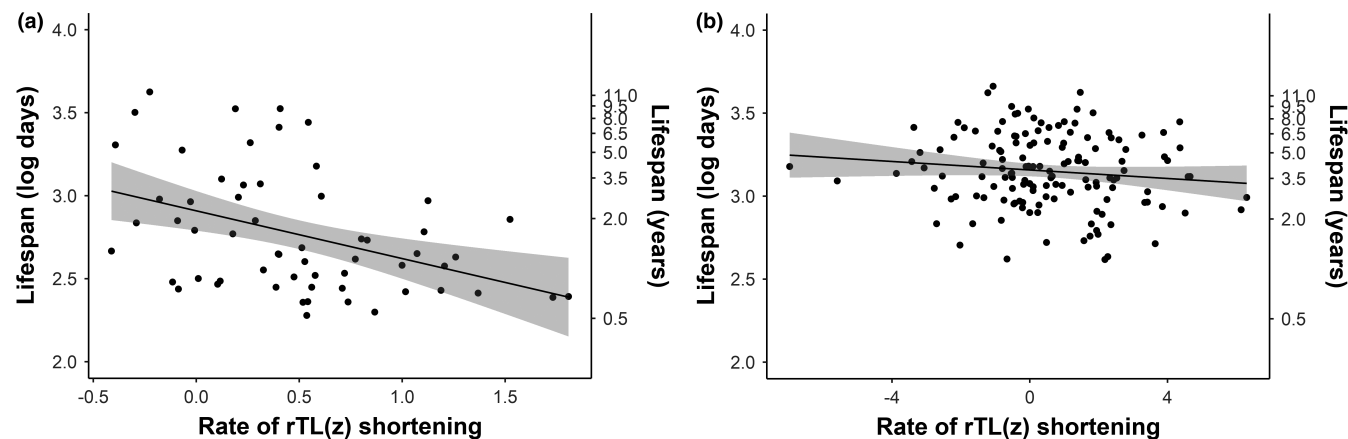
FIGURE 2 (a) Lifespan in relation to the rate of telomere shortening in the first year of life. Shortening rates are expressed as *z*-transformed rTL values per log-transformed time in days and negative shortening rates indicate telomere "elongation." Lifespan in days was log transformed (left *y*-axis), and for ease of interpretation is shown on a linear scale on the right *y*-axis. (b) No association between the rate of telomere (rTL(*z*)) shortening (per log day) during later life (*x*-axis, negative values indicate telomere "elongation") and lifespan; lifespan is shown in log days on the left *y*-axis and in years on the right *y*-axis

of death does remain complex (Campisi, 2003; Kurz et al., 2004), our results suggest that telomere shortening rate quite convincingly acts as a biomarker of the early life experiences related to mortality risk an individual has faced.

Across adulthood, neither the rate of telomere shortening nor absolute telomere length was predictive of lifespan in our study. Sample size in this later life analysis was considerably larger than in the first year analysis, and lack of power is therefore unlikely to explain this result. Our finding contrasts with results from Barrett et al. (2013), where the rate of telomere shortening and absolute telomere length in adulthood predicted lifespan in adult Seychelles warblers. This difference may (partly) reflect the fact that, in contrast to Barrett et al. (2013), telomere length in our study did not significantly change in adulthood (from late in the first year to various time points thereafter). Relatively little is still known of the predictive capacity of telomere shortening on lifespan, and more studies are needed to establish if and how this predictive capacity generally changes through life.

4.2 | Lifespan and telomere length across life

In a previous study of the same population of purple-crowned fairy-wrens, Eastwood et al. (2019) showed a strong correlation between nestling telomere length (at ~7 days) and lifespan in birds that survived to at least 1 year old (i.e., a similar set of individuals as that included here). In the present study, we tested for an association between lifespan and telomere length measured at two time points in the first year of life, and our results confirm our earlier finding, with longer telomere length during the first year associated with longer lifespan, although the relationship was less strong. With increasing age, environmental effects on telomere length will accumulate, diluting early life genetic and environmental effects. This may explain why the strength with which telomere length

TABLE 2 Telomere length after the first year of life in relation to age and lifespan

Dependent variable: Relative telomere length (rTL(*z*))			
Fixed effects	**Estimate (SE)**	***t*-value**	***p*-value**
Intercept	0.441 (0.882)	0.501	.618
Lifespan	0.410 (0.410)	1.000	.319
Lifespan × Delta age	0.181 (0.679)	0.266	.790
Delta age	−1.008 (2.222)	−0.454	.650
Average age	−0.781 (0.508)	−1.537	.127
Sex (male)	0.191 (0.119)	1.609	.110
Storage buffer (Longmire)	0.391 (0.233)	1.682	.095
Days between sampling and DNA extraction (*z*)	−0.163 (0.109)	−1.488	.142
Days between DNA extraction and telomere quantification (*z*)	1.167 (1.050)	1.111	.267
Random effects	**Variance (SD)**		
Individual ID	0.053 (0.230)		
qPCR run ID	0.067 (0.258)		
Full sibling ID	0.221 (0.470)		
Hatch year	0.018 (0.135)		
Residual	0.603 (0.777)		
	Marginal R^2	**Conditional R^2**	
	.031	.393	

Note: All age parameters are expressed in log(days) and were log-transformed prior to the calculation of average and delta age. Note that because delta age was mean centred, the estimate of the lifespan effect remains informative despite being included in an interaction term.

Abbreviations: SE, standard error; SD, standard deviation.

predicts lifespan declines later in life, being undetectable in adulthood (Table 2), relatively weak across the first year of life (Table 1) and strongest in nestlings (Eastwood et al., 2019). This suggestion is supported by the observation that variance in telomere length associated with full sibling ID, which reflects a mixture of genetic and other early life effects, was much smaller later in life (7%) than in the first year of life (33%) (compare Tables 1 and 2). This finding is opposite to two recent longitudinal studies in wild seabirds (*Sterna hirundo*) (Vedder et al., 2021) and Soay sheep (*Ovis aries*) (Froy et al., 2021) that showed strong, positive genetic correlations of adult telomere length with lifespan (but no effect of telomere shortening). More longitudinal studies of telomere length and shortening across life are clearly required to tease apart the relative significance of inherited and environmental effects on the relationship between telomeres and lifespan, and how this may vary between species.

In conclusion, our study lends strength to the view that telomere length measured very early in life (during development) or longitudinal assessments of telomere shortening during the first year of life may constitute more useful biomarkers of total life expectancy than either telomere length after development or maturation are complete, or telomere shortening later in adulthood. More broadly, our study supports the notion of telomere shortening as an indicator of senescence/mortality risk, rather than a causal mechanism. Future work is needed to assess the generality of this result for wild populations of animals and to tease apart the relative contributions from heritable and environmental effects on telomere length and telomere length dynamics (which recent research has started to address: Froy et al., 2021; Vedder et al., 2021). This will help to develop our understanding of the role of telomere dynamics in driving the evolution of life histories.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

E.L.S., S.V. and A.P. formulated the research question, E.L.S., N.T., M.J.R., N.H.A., M.F., M.L.H., S.A.K. and A.P. contributed to sample collection in the field, J.R.E. performed the qPCR analyses and analysed data, and E.L.S. analysed the data and wrote the manuscript with input from A.P. and S.V. and co-authors.

OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://doi.org/10.6084/m9.figshare.14527017.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within its Appendix S1 and have also been archived in the publicly accessible repository FigShare (https://doi.org/10.6084/m9.figshare.14527017).

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